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(54) Chemically modified surface for large molecule attachment.

(57) The attachment and/or orientation of biologically active large molecules can be controlled by varying the surface chemistry of a substrate in contact with the large molecule. The surface chemistry of polymeric surfaces can be varied by using selected homopolymers, copolymerization, surface grafting, chemical modification and plasma treatment of the surface. In particular, plasma modification processes may be used to provide specific chemical functional groups, element ratios and other surface properties.

CHEMICALLY MODIFIED SURFACE FOR
LARGE MOLECULE ATTACHMENT

The present invention relates generally to methods and articles for influencing the attachment of large molecules to substrate surfaces. More particularly, the invention relates to the modification of the surfaces of organic and inorganic substrates so as to provide a chemically modified surface which influences the orientation and attachment of large molecules having available functional groups, such as proteins, to substrate surfaces.

It is demonstrated herein that the orientation and attachment of large molecules on polymer surfaces can be selectively controlled by varying the surface chemistry of the substrate in contact with the large molecule.

In a preferred embodiment of the present invention, the surface chemistry of a substrate is modified by subjecting the substrate to a plasma of a suitable material. It is known to subject substrates to plasmas of various types for accomplishing various purposes. A brochure of Tegal Corporation, CP1287, for example indicates that plasmas can be used for dry stripping, cleaning and etching of electronic parts, cleaning optical surfaces, preparing surfaces having bonding, wettability and chemical resistance properties and dry cleaning of substrates to improve film deposition and adherence. It is not known, however, that the attachment and orientation of

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large molecules can be influenced by chemical modification of the surface of the substrate on which the molecule is attached.

5 The importance of the ability to control the attachment of large molecules (either to enhance or hinder such attachment) is self evident. The importance of being able to control the orientation of attachment can be illustrated by reference to a particular large molecule, such as an antibody.

10 Antibodies are large protein molecules having a Y configuration. The two short arms of the Y (F_{ab}) contain the immunologically active sites which are capable of recognizing an antigen. For some immunological assays it is desirable to couple the antibody

15 to a substrate. Such coupling must take place predominantly by attachment of the long arm of the Y (F_c) antibody configuration to retain the immunologic activity of the antibody.

20 Other examples of large molecules having available functional groups which are sometimes attached to a substrate wherein control of attachment and orientation are important include: proteins bound to a column for purification of other proteins by column elutriation, immobilization of enzymes in a

25 biologically active mode and binding of hormones for biological treatment.

30 Accordingly, it is a principal object of the present invention to provide chemically specific surfaces for influencing the attachment and/or orientation of large molecules having available functional groups onto the surface.

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It is another object of the present invention to provide a substrate with a chemically modified surface which influences the attachment and/or orientation of large molecules onto the surface.

5 It is a further object of the present invention to provide a substrate with a chemically modified surface controls the attachment and orientation of antibodies onto the substrate surface.

10 It is a still further object of the present invention to provide a method and an article for controlling the attachment and/or orientation of biologically active large molecules having available functional groups onto the surface of the article.

15 These and other objects of the present invention will become more apparent from the accompanying drawings wherein:

Fig. 1 is a schematic diagram of an apparatus which can be employed in the practice of the invention.

20 Fig. 2 is a perspective view of a sample holder illustrated in the apparatus of Fig. 1.

Fig. 3 is a schematic representation of an antibody.

25 Figs. 4 through 6 are plots of the attachment and orientation characteristics of various untreated substrates and substrates treated in accordance with the invention.

30 In general, it has been discovered that the attachment and/or orientation of biologically active large molecules can be controlled by varying the

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surface chemistry of a substrate surface in contact with the large molecule. The surface chemistry of a polymeric surface can be varied by using selected homopolymers, copolymerization, surface grafting and chemical modification, and by plasma treatment of the surface. In particular, plasma modification processes may be used to provide specific chemical functional groups, elemental ratios and other surface properties. In accordance with the invention, polymeric surfaces were prepared by plasma modification techniques which yielded a range of surface chemistries and properties. The modified polymeric surfaces were subjected to solutions of biologically active large molecules and subsequently tested to demonstrate that attachment and orientation of the large molecule is highly dependent on surface chemistry.

As used herein, the term "large molecule" means any organic or inorganic molecule having a molecular weight greater than about 1,000g/molecular. The term "available functional group" means a chemically active functional group contained in a large molecule which is available for binding to a substrate. Suitable functional groups include amino, carboxyl, sulfhydryl, hydroxyl, sulfate, alkyl-halides, to name a few. The term "biologically active large molecule" means an amino acid containing molecule (usually a protein) which has biological activity.

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A plasma of a suitable material may be used to modify the surface layer of organic and inorganic substrates, including polymeric materials, glass and metals, regardless of thickness and which is independent of substrate composition, so as to provide specific chemical functional groups on the surface of the substrate. In the case of glass and metals, it is desirable to include a carbon source, such as methane, for example, to provide a thin organic layer on the inorganic substrate. Such plasma process is suitable for providing a surface for any cellular growth application whether the desired goal is to enhance or retard cell growth. The surface of the substrate is irreversibly modified by grafting specific chemical functional groups onto the surface with a plasma of a suitable material or with selected components of a plasma of a suitable material. Suitable materials include, but are not limited to oxygen, carbon, hydrogen, nitrogen, halogen, sulfur, phosphorus, mixtures thereof and compounds thereof. The surfaces which are modified in accordance with the present invention have specific chemical functional groups grafted onto the substrate surface and provide suitable surface chemistry for tissue culture growth or suppression of growth.

In another embodiment of the present invention there is provided a process for modifying the surface of a substrate, specifically the modification of organic polymeric materials by treatment with specific chemical species comprising:

Providing a substrate within a reaction zone.

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Providing a plasma of a suitable material in the reaction zone which includes neutral material, positive ions of the material, negative ions of the material, electrons and photons.

5 Preventing at least one of the components of the plasma from contacting the surface of the substrate in the reaction zone.

10 Contacting the surface of the substrate with the remainder of the components of the plasma in the reaction zone, and

Forming specific functional groups of the material on the surface of the substrate.

15 This embodiment of the present invention is based on the discovery that utilization of selected reactive species of a plasma in contact with a substrate can be employed to provide specific chemical functional groups on the surface of the substrate which act to influence the attachment and/or orientation of large molecules.

20 Referring now to the figures, other features and advantages of the present invention will be described.

As shown in Fig. 1 there is provided a first gas reservoir 11, a second gas reservoir 13 with conduit means 15 to deliver either or both a first gas and a second gas to a vacuum chamber 17. Flow meters 19 and 21 are provided for measuring gas flow rate and a vacuum gauge 23 is provided to monitor the pressure within the vacuum chamber 17. Valves 25, 27 and 29 are provided to regulate the flow rate of the gas in the first gas reservoir 11, the second gas

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reservoir 15 and the gas entering the vacuum chamber 17. Prior to use, the vacuum chamber 17 is evacuated by opening valve 31 to vacuum pump 33. Suitable electrodes 35 and 37 are connected to a suitable voltage source 39. The reactor system also includes a trap 41, vent conduit 43 and its valve 45.

As best seen in Fig. 2, a substrate 47 to be treated in accordance with the invention is placed in a sample holder 55 and disposed within the vacuum chamber 17. The sample holder includes a grid assembly of three grids, designated grid I, grid 2 and grid 3. A collector 49 which can be electrically biased is disposed on the other side of the sample from the grid system. The sample is held in place between grid 3 and the collector by means of a suitable holder 51.

In operation, any or none of the three grids or the collector can be biased with positive or negative charge to repel selective components of a plasma generated between the electrodes 35 and 37. The substrate in the sample holder is then subjected to reaction with those components of the plasma which are not repelled.

The present invention can be employed to alter the surfaces of solid polymeric materials, including natural and synthetic addition and condensation polymers. Such polymeric materials include polyolefins, such as polyethylene, polypropylene, polyisobutylene and ethylene-alpha-olefin copolymers, acrylic polymers and copolymers, such as polyacrylate, polyethylmethacrylate, polyethylacrylate;

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vinyl halide polymers and copolymers, such as poly-
vinyl chloride; polyvinyl ethers, such as polyvinyl
methyl ether; polyvinylidene halides, such as poly-
vinylidene fluoride and polyvinylidene chloride;
5 polyacrylonitrile, polyvinyl ketones; polyvinyl
amines; polyvinyl aromatics, such as polystyrene,
polyvinyl esters such as polyvinyl acetate; copolymers
of vinyl monomers with each other and olefins, such
as ethylene-methyl methacrylate copolymers, acryloni-
10 trile-styrene copolymers, ABS resins, and ethylene-
vinyl acetate copolymers; natural and synthetic
rubbers, including butadiene-styrene copolymers,
polyisoprene, synthetic polyisoprene, polybutadiene,
butadiene-acrylonitrile copolymers, polychloroprene
15 rubbers, polyisobutylene rubber, ethylene-propylene
rubber, ethylene-propylene-diene rubbers, isobuty-
lene-isoprene copolymers and polyurethane rubbers;
polyamides, such as Nylon 66 and polycaprolactam;
polyesters such as polyethylene terephthalate, alkyd
20 resins; phenol-formaldehyde resins; urea-formaldehyde
resins, melamine-formaldehyde resins; polycarbonates;
polyoxymethylenes; polyimides; polyethers; epoxy
resins, poly-urethanes; wool; cotton; silk; rayon;
rayon-triacetate; cellulose; cellulose acetate,
25 cellulose butyrate; cellulose acetatebutyrate;
cellophane; cellulose nitrate; cellulose propionate;
cellulose ethers; and carboxymethyl cellulose.

Inorganic materials, the surfaces of which can
be modified in accordance with the invention, include
30 non-metals, such as graphite; metals, such as iron,
aluminum, tin, copper and nickel; metal and other

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5 elemental oxides; such as magnesium oxide, silica, alumina and titania; minerals, such as clay, pyrite, and asbestos; salts, such as sodium chloride and calcium carbonate; and such synthetic compositions such as glass and refractories.

10 The substrates whether organic or inorganic, can be any shape, such as continuous or particulate, porous or impervious, and large or small. The invention can be employed for altering the surfaces of crystals, powders, plates, strips, films, sheets, wire, fibers, fabrics, filaments, tubing, and cast, extruded or compressed articles, and the like.

15 Plasmas of the elements of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, the halogens, mixtures thereof and compounds thereof are useful to modify the surface of substrates in accordance with the present invention.

20 Four types of plasmas can be generated; these are thermal plasmas, discharge plasmas, beam plasmas (such as ion beams and electron beams) and hybrid plasmas such as corona discharges. Suitable plasmas for use in the method of the present invention are discharge plasmas, beam plasmas and hybrid plasmas. Suitable plasmas can be generated by use of DC
25 sources or AC sources having a frequency through the microwave range at power levels from about 1.0W to about 10 kW. Although not limited to high frequency sources, such as radiofrequency (r.f.) sources, plasmas of this type are particularly useful for the
30 method and articles of the present invention in that selected plasma species concentrations can be controlled by source frequency, power, and system

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pressure. It is also true that r.f. plasmas are suitable for uniformly modifying the surface of more complex article shapes, such as tubing and other enclosures, for which DC and low frequency generated plasmas are not satisfactory.

In general, the r.f. plasmas suitable for use in the present invention are generated at a frequency of from about 1.0 to about 300 MHz at a power to initiate breakdown, such as from about 5 to about 1000 watts at pressures ranging from 0.001 to 10 Torr. The articles are usually subjected to the r.f. plasma for a period of from about 0.1 sec to about 120 minutes, although longer and shorter periods can be used. The plasma treatment can be followed by a quench cycle designed to provide an atmosphere suitable for reacting with residual active polymer species at or near the surface with pressures ranging from 1 Torr to 760 Torr for time periods of 1 second to 4 hours. The r.f. plasma can be generated between two flat plates, as shown in Figure 1, or can be generated with a helical coil, as shown in United States Patent No. 4,188,426.

The following examples further illustrate various features of the invention but are not intended to in any way limit the scope of the invention which is set forth in the appended claims.

EXAMPLE I

It is well known to test for small concentrations of antigens using antibodies and radiolabeling techniques. This technique is generally referred to

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as RIA (radioimmunoassay) and is widely used commercially. A specialized form of RIA referred to as solid phase RIA relies on the ability of a polymer to bind the antibody. When an antigen is brought
5 into proximity to the polymer, the antigen reacts with the bound antibody. A competitive assay can be developed by adding a known amount of labeled antigen to the tube. The method, however, is highly dependent on the immunologic activity of the bound
10 antibody maintained on the polymer surface.

In the present invention, a method is disclosed that allows control of the surface concentration of antibody binding and the orientation of the antibody molecule on the substrate surface by plasma surface
15 modification processes.

The chemical definitions of the IgG antibody has evolved to designate the F_c fragment and F_{ab} fragments separately. The immunologically active sites of the antibody reside in the F_{ab} fragments
20 as illustrated in the attached diagram of the IgG molecule contained in Figure 3. If an antibody is coated onto a surface, the F_{ab} fragments must be accessible for binding antigen. Therefore, the preferred orientation for binding IgG to a solid
25 substrate is with the F_c fragment attached to the substrate leaving the two F_{ab} fragments accessible for binding.

Polystyrene molded strips were placed in the vacuum chamber of the apparatus of Fig. 1. The
30 vacuum chamber 17 was subsequently evacuated to 50 μ

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for 20 minutes and then filled to 200 μ with SO₂ for 10 minutes. A 10 MHz r.f. plasma was operated for five minutes with 45 watts power. The vacuum chamber 17 was maintained at 200 μ of SO₂ for two minutes, 5 pumped down to 50 μ for two minutes, and then exposed to air at atmospheric pressure. The relative zeta potential of the surface of the polystyrene strips was determined to be -19.4 mV. The surface tension as measured by Wilhelmy plate contact angle was 10 determined to be 72° \pm 3°.

To measure antibody binding to the modified surface, purified (ammonium sulfate fractionation) rabbit IgG was radiolabeled with ¹²⁵I. The amount of IgG bound was measured at ambient temperature as a 15 function of pH of the coating solution comprised of 10 μ g/ml IgG in phosphate buffered saline with ionic strength of 0.156 M. The surface of polystyrene strips (both treated and untreated by the plasma process described above) was exposed to the solution 20 for 60 minutes and centrifuged dry. The specific activity of the labeled IgG was 2.2 x 10³ DPM/ μ g. The results of the antibody binding experiment are illustrated in Figure 4a. At low pH the amount of antibody bound has increased by 50%.

25 For the measurement of immunologic activity to T₄ the surface was exposed to a solution of T₄-antiserum purified by affinity column chromatography. The T₄-antiserum solution concentration used was 100 μ g/ml in phosphate buffered saline with ionic 30 strength of 0.156 M. The surface of the polystyrene strips was exposed to the antiserum solution for

20 minutes and centrifuged dry. The coated surface was then exposed to radiolabeled T_4 solution for 45 minutes at room temperature and rinsed under running water.

5 The results of the T_4 measurement are given in Figure 4b. At low pH the amount of antigen bound to plasma treated polystyrene strip was increased by 55% as compared to the untreated polystyrene strip.

10

EXAMPLE 2

Polystyrene molded strips were placed into the vacuum chamber 17 which was evacuated to 75μ for 10 minutes and then brought up to a pressure of 180μ of NH_3 for a period of two minutes. A 10 MHz r.f. plasma was operated for five minutes at a power of 35 watts. The chamber was then exposed to air at atmospheric pressure. The measured relative zeta potential was +8.2 mV. The Wilhelmy plate contact angle was determined to be $60^\circ \pm 2^\circ$.

To measure antibody binding to the modified surface, purified (ammonium sulfate fractionation) rabbit IgG was radiolabeled with ^{125}I . The amount of IgG bound was measured at ambient temperature as a function of pH of the coating solution comprised of 10 $\mu\text{g/ml}$ IgG in phosphate buffered saline with ionic strength of 0.156 M. The surface of treated and untreated polystyrene strips was exposed to the solution for 60 minutes and centrifuged dry. The specific activity of the labeled IgG was 2.2×10^3 DPM/ μg . The results of the antibody binding experiment are illustrated in Figure 5a. The maximum increase in the amount of antibody bound at any pH is 25%.

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For the measurement of immunologic activity to T_4 the surface was exposed to a solution of T_4 -antiserum purified by affinity column chromatography. The T_4 -antiserum solution concentration used was 100 $\mu\text{g/ml}$ in phosphate buffered saline with ionic strength of 0.56 M. The surface of the polystyrene strips was exposed to the antiserum solution for 20 minutes and centrifuged dry. The coated surface was then exposed to radiolabeled T_4 solution for 45 minutes at room temperature and rinsed under running water.

The results of the T_4 study are given in Figure 5b. It is evident from the data that there is a significant decrease in T_4 bound to the plasma treated polystyrene strips even though an increase in antibody binding was measured. This data indicates that fewer F_{ab} sites are exposed and illustrates that the orientation of the IgG has been significantly affected by the surface treatment.

20

EXAMPLE 3

Polystyrene molded strips were placed into a vacuum chamber 17 which was evacuated to 75 μ for 10 minutes and then brought up to a pressure of 180 μ of NH_3 for a period of two minutes. A 10 MHz r.f. plasma was operated for five minutes at a power of 35 watts. The chamber was then brought up to a 700 Torr pressure of CO_2 for a two-hour quench cycle. The plasma chamber was pumped down to 1 Torr and then opened to air at atmospheric pressure. The relative

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zeta potential of this surface was measured as +31.3 mV. The Wilhelmy plate contact angle was determined to be $64^{\circ} \pm 3^{\circ}$.

To measure antibody binding to the modified surface, purified (ammonium sulfate fractionation) rabbit IgG was radiolabeled with ^{125}I . The amount of IgG bound was measured at ambient temperature as a function of pH of the coating solution comprised of 10 $\mu\text{g/ml}$ IgG in phosphate buffered saline with ionic strength of 0.156 M. The surface of treated and untreated polystyrene strips was exposed to the solution for 60 minutes and centrifuged dry. The specific activity of the labeled IgG was 2.2×10^3 DPM/ μg . The results of the antibody binding experiment are illustrated in Figure 6a. The maximum increase in IgG bound to the surface at any pH is 20%.

For the measurement of immunologic activity to T_4 the surface was exposed to a solution of T_4 -antiserum purified by affinity column chromatography. The T_4 -antiserum solution concentration used was 100 $\mu\text{g/ml}$ in phosphate buffered saline with ionic strength of 0.156 M. The surface of the polystyrene strips was exposed to the antiserum solution for 20 minutes and centrifuged dry. The coated surface was then exposed to radiolabeled T_4 solution for 45 minutes at room temperature and rinsed under running water.

The results of the T_4 study are given in Figure 6b. It is evident from the data that there

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5 is a significant decrease in T_4 bound to the plasma treated polystyrene strips even though an increase in IgG antibody binding was measured. This data further substantiates that the orientation of the IgG antibody molecule has been significantly affected by the surface treatment.

WHAT IS CLAIMED IS:

1 1. A method for influencing the attachment
2 and/or orientation of large molecules on a substrate
3 comprising modifying the surface chemistry of said
4 substrate by subjecting the surface of said substrate
5 to a plasma which is produced from a material se-
6 lected from the group consisting of carbon, hydrogen,
7 oxygen, nitrogen, sulfur, phosphorus, halogens and
8 compounds thereof.

1 2. A method in accordance with Claim 1 wherein
2 said plasma is a discharge plasma generated at a
3 frequency of from about 1 to about 300 megahertz at a
4 power of from about 5 to about 1000 watts at a
5 pressure of from about 0.001 to about 10 Torr.

1 3. A method in accordance with Claims 1 and 2
2 wherein said plasma material is selected from oxygen
3 and oxygen compounds.

1 4. A method in accordance with Claims 1 and 2
2 wherein said plasma material is selected from nitro-
3 gen and nitrogen compounds.

1 5. A method in accordance with Claims 1 and 2
2 wherein said plasma material is selected from sulfur
3 and sulfur compounds.

1 6. A method in accordance with Claims 1 and 2
2 wherein said plasma material is selected from phos-
3 phorus and phosphorus compounds.

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1 7. A method in accordance with Claims 1 and 2
2 wherein said plasma material is selected from halogen
3 and halogen compounds.

1 8. A method in accordance with Claims 1 and 2
2 wherein said plasma material is selected from carbon
3 and carbon compounds.

1 9. A method in accordance with Claims 1 and
2 2 wherein said plasma is selected from hydrogen and
3 hydrogen compounds.

1 10. A method in accordance with Claim 1 wherein
2 said large molecule is a protein.

1 11. A method in accordance with Claim 1 wherein
2 said large molecule is an antibody.

1 12. A method in accordance with Claim 1 wherein
2 said large molecule is an antigen.

1 13. An article suitable for influencing the
2 attachment and/or orientation of large molecules
3 thereto comprising a polymeric article whose surface
4 chemistry has been modified by subjecting the surface
5 of said polymeric article to a plasma which is
6 produced from a material selected from the group
7 consisting of carbon, hydrogen, oxygen, nitrogen,
8 sulfur, phosphorus, halogens and compounds thereof.

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1 14. An article in accordance with Claim 13
2 wherein plasma is a discharge plasma generated at a
3 frequency of from about 1 to about 300 megahertz at a
4 power of from about 5 to about 1000 watts at a
5 pressure of from about 0.001 to about 10 Torr.

1 15. An article in accordance with Claims 13 and
2 14 wherein said plasma material is selected from
3 oxygen and oxygen compounds.

1 16. An article in accordance with Claims 13 and
2 14 wherein said plasma material is selected from
3 nitrogen and nitrogen compounds.

1 17. An article in accordance with Claims 13 and
2 14 wherein said plasma material is selected from
3 sulfur and sulfur compounds.

1 18. An article in accordance with Claims 13 and
2 14 wherein said plasma material is selected from
3 phosphorus and phosphorus compounds.

1 19. An article in accordance with Claims 13 and
2 14 wherein said plasma material is selected from
3 halogen and halogen compounds.

1 20. An article in accordance with Claims 13
2 and 14 wherein said plasma material is selected from
3 carbon and carbon compounds.

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1 21. An article in accordance with Claims
2 13 and 14 wherein said plasma material is selected
3 from hydrogen and hydrogen compounds.

1 22. An article in accordance with Claim 13
2 wherein said large molecule is a protein.

1 23. An article in accordance with Claim 13
2 wherein said large molecule is an antibody.

1 24. An article in accordance with Claim 13
2 wherein said large molecule is an antigen.

1 25. An article suitable for influencing the
2 attachment and/or orientation of large molecules
3 thereto comprising an inorganic article whose surface
4 chemistry has been modified by subjecting the surface
5 of said inorganic article to a plasma which is
6 produced from a material selected from the group
7 consisting of carbon, hydrogen, oxygen, nitrogen,
8 sulfur, phosphorus, halogens and compounds thereof.

1 26. An article in accordance with Claim 25
2 wherein plasma is a discharge plasma generated at a
3 frequency of from about 1 to about 300 megahertz at a
4 power of from about 5 to about 1000 watts at a
5 pressure of from about 0.001 to about 10 Torr.

1 27. An article in accordance with Claims 25 and
2 26 wherein said plasma material is selected from
3 oxygen and oxygen compounds.

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1 28. An article in accordance with Claims 25 and
2 26 wherein said plasma material is selected from
3 nitrogen and nitrogen compounds.

1 29. An article in accordance with Claims 25 and
2 26 wherein said plasma material is selected from
3 sulfur and sulfur compounds.

1 30. An article in accordance with Claims 25 and
2 26 wherein said plasma material is selected from
3 phosphorus and phosphorus compounds.

1 31. An article in accordance with Claims 25 and
2 26 wherein said plasma material is selected from
3 halogen and halogen compounds.

1 32. A method for influencing the attachment
2 and/or orientation of large molecules on a substrate
3 comprising modifying the surface chemistry of said
4 substrate by a method comprising;

5 (a) providing said substrate within a reaction
6 zone

7 (b) introducing a material into said reaction
8 zone, said material being suitable for providing a
9 plasma

10 (c) subjecting said material to plasma pro-
11 ducing conditions, whereby the plasma comprises
12 neutral material, positive ions of said material,
13 negative ions of said material, electrons and photons

14 (d) preventing at least one of the components
15 of said plasma from contacting the surface of said
16 substrate,

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17 (e) contacting the surface of said substrate
18 with the remainder of said components of said plasma,
19 and
20 (f) forming specific functional groups of said
21 material on the surface of said organic substrate.

1 33. A method in accordance with Claim 32
2 wherein said plasma is produced under electrical
3 discharge conditions.

1 34. A method in accordance with Claims 32 and
2 33 wherein said material is selected from oxygen,
3 nitrogen, halogen, sulfur, compounds thereof and
4 mixtures thereof.

1 35. A method in accordance with Claims 32 and
2 33 wherein said vaporized material is oxygen and
3 compounds thereof.

1 36. A method in accordance with Claims 32 and
2 33 wherein said vaporized material is nitrogen and
3 compounds thereof.

1 37. A method in accordance with Claims 32 and
2 33 wherein said vaporized material is halogen and
3 compounds thereof.

1 38. A method in accordance with Claims 32 and
2 33 wherein said vaporized material is sulfur and
3 compounds thereof.

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1 39. A method in accordance with Claim 32
2 wherein said large molecule is a protein.

1 40. A method in accordance with Claim 32
2 wherein said large molecule is an antibody.

1 41. A method in accordance with Claim 32
2 wherein said large molecule is an antigen.

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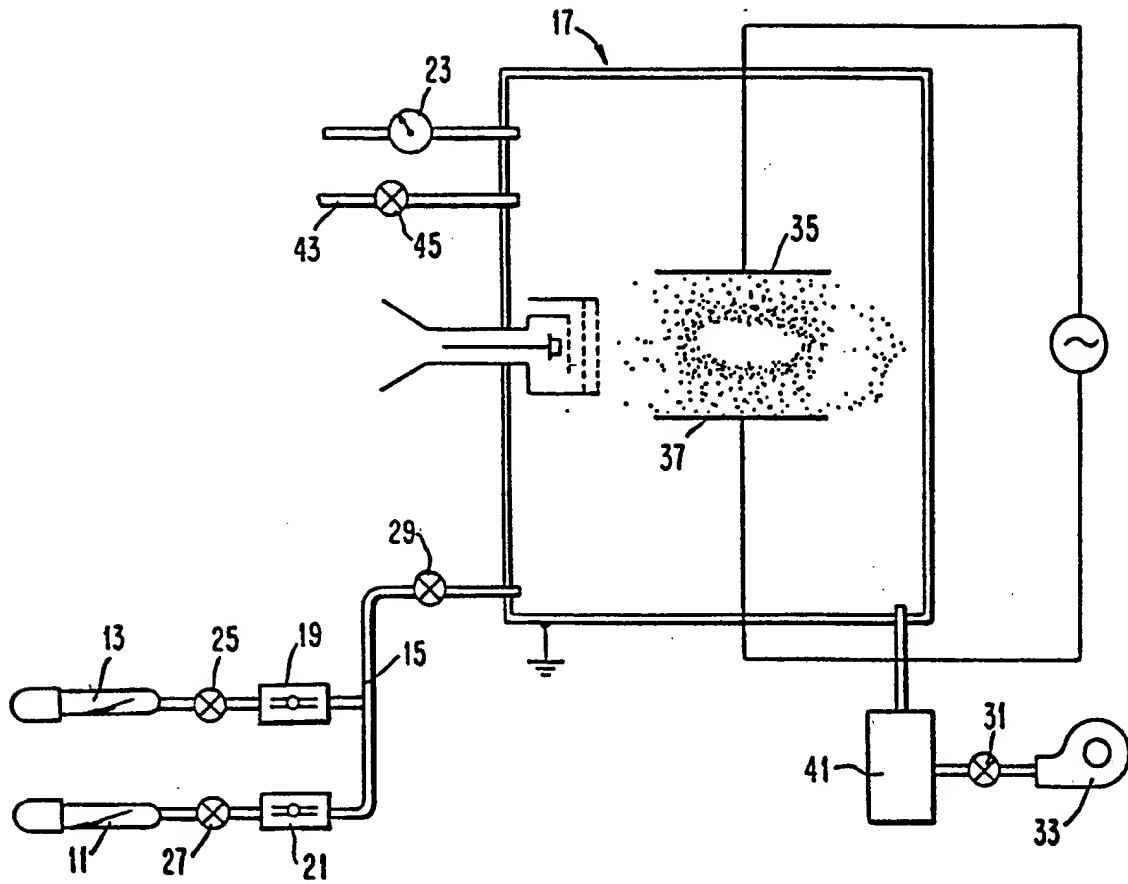


FIG. 1.

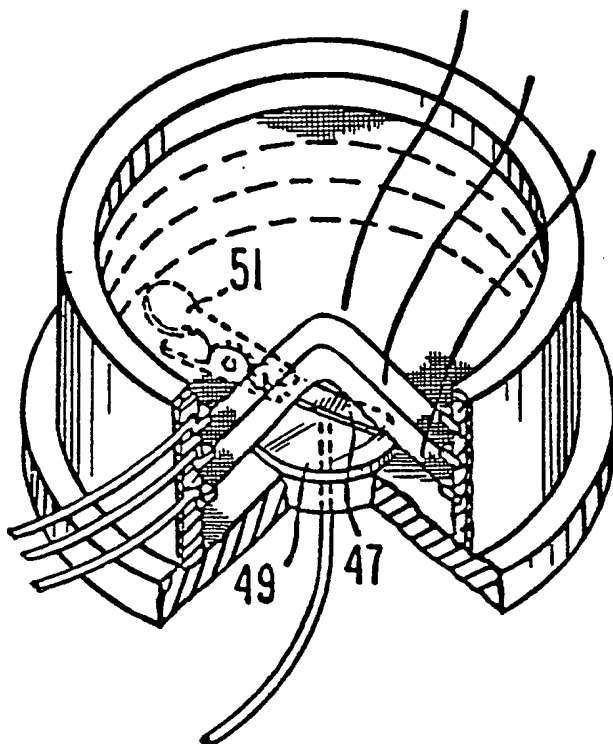


FIG. 2

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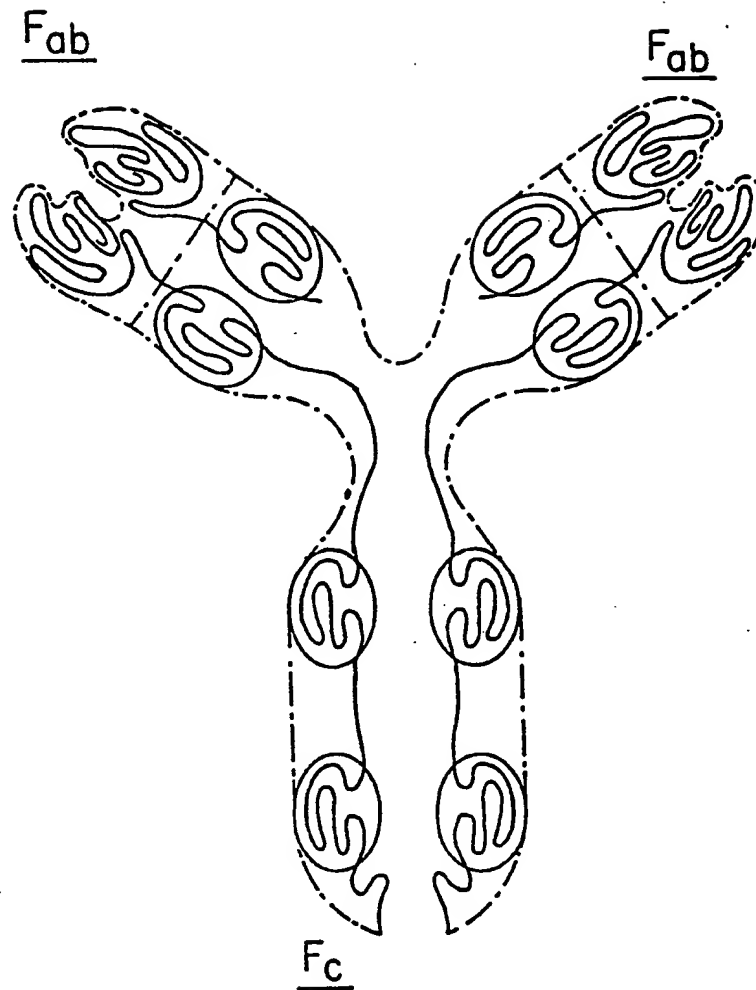


FIG. 3

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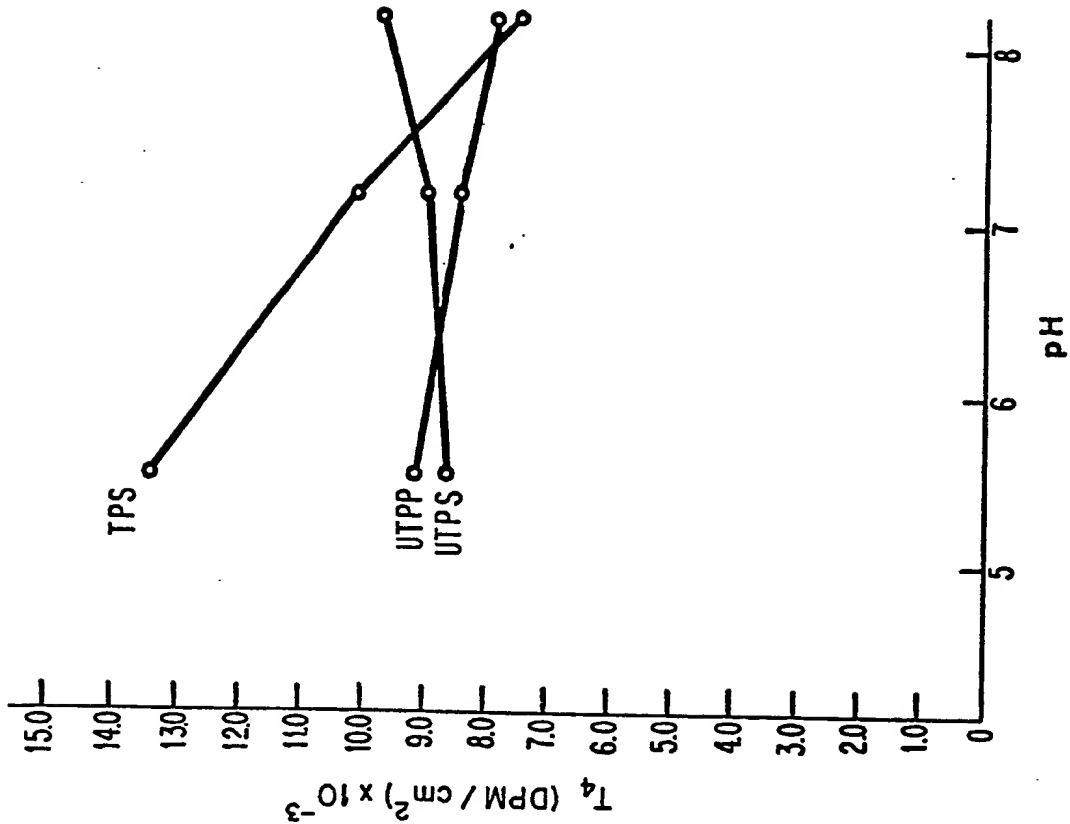


FIG. 4B

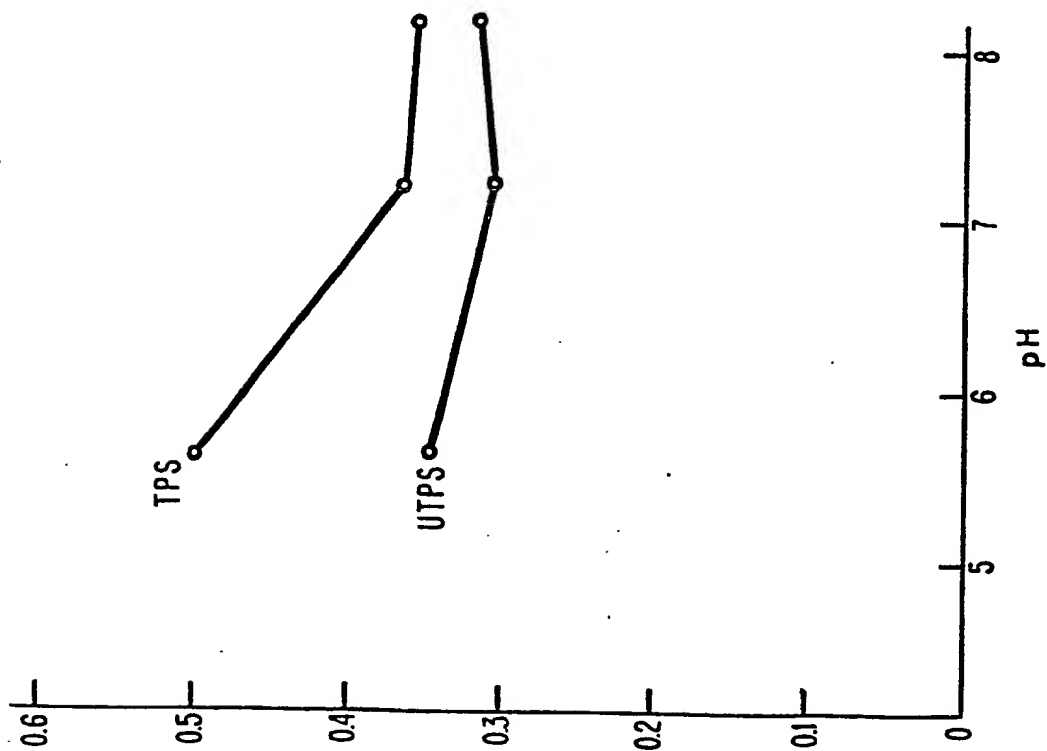


FIG. 4A

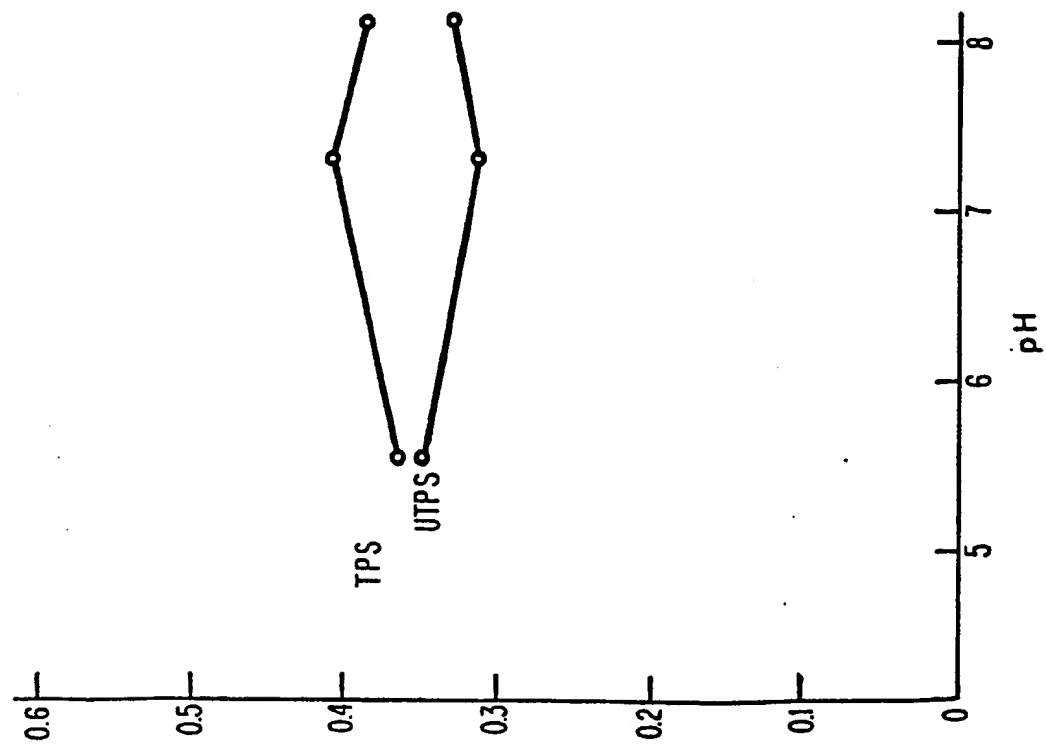


FIG. 5A

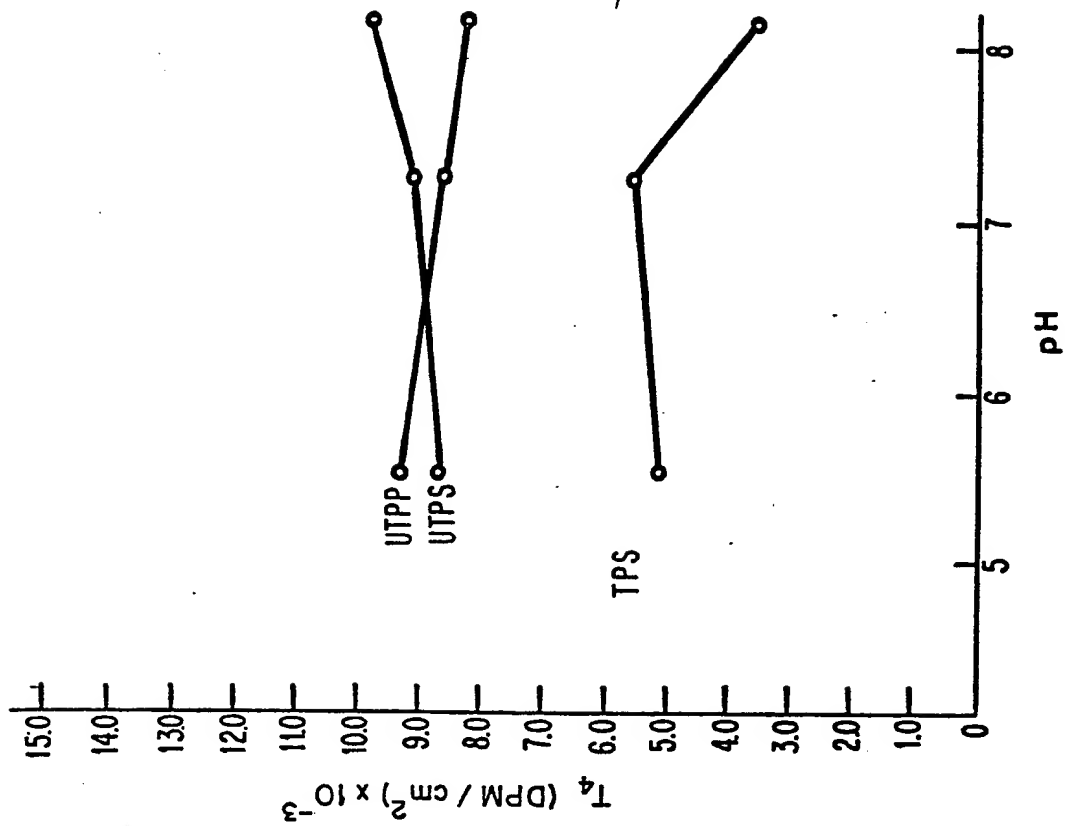
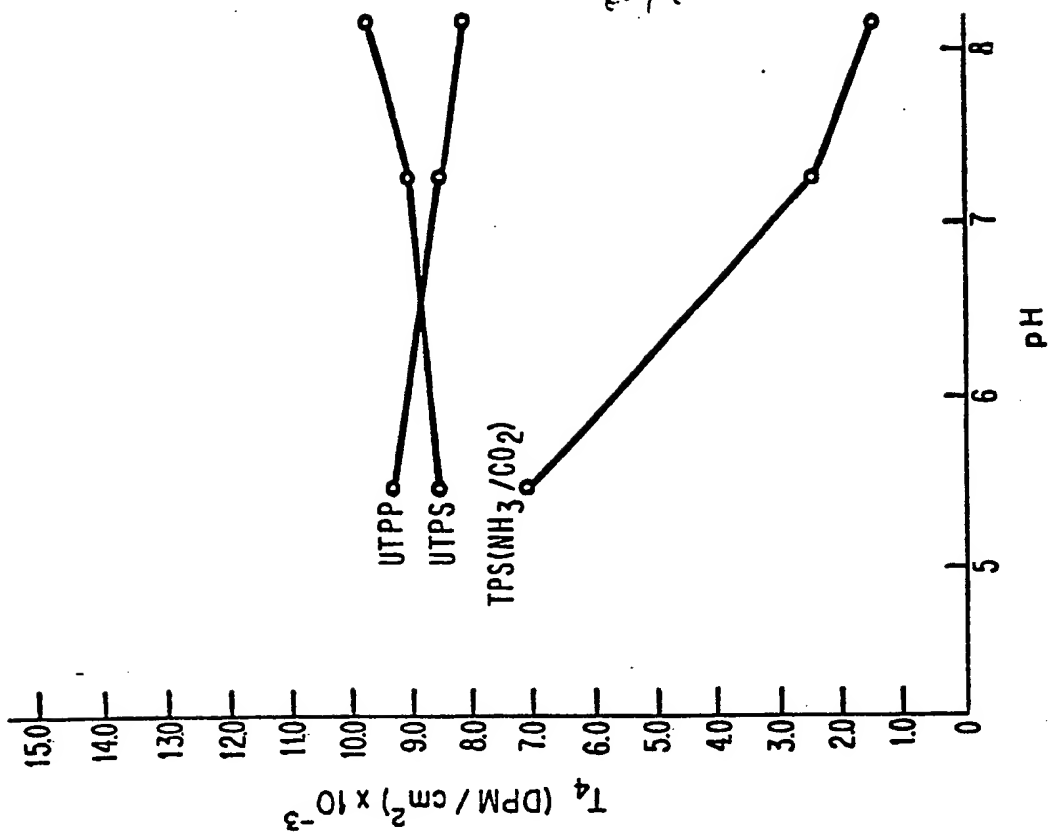
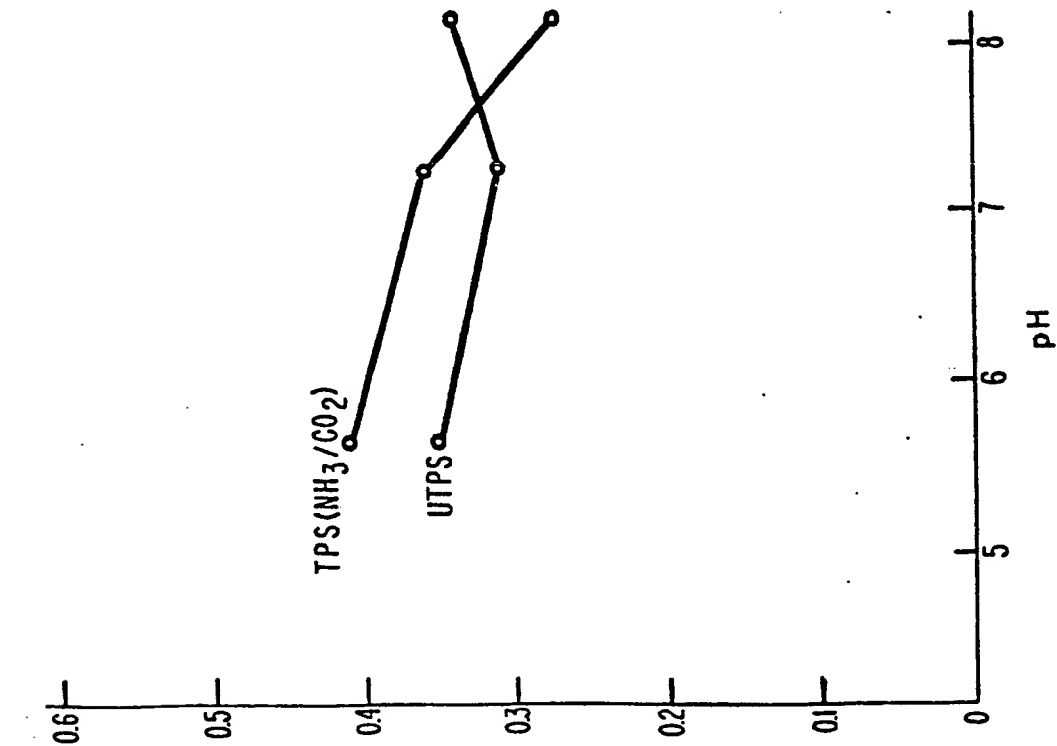


FIG. 5B

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European Patent
Office

EUROPEAN SEARCH REPORT

0104608

Application number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 83109418.0
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 7)
A, D	<p>US - A - 4 188 426 (AUERBACH)</p> <p>* Abstract; claims 1,2; column 4, line 68 - column 5, line 2 *</p> <p>--</p>	1, 2, 7-9, 32	<p>C 12 N 11/00</p> <p>B 01 J 19/08</p> <p>C 12 N 11/08</p> <p>C 12 N 11/14</p>
A	<p>EP - A1 - 0 046 613 (REGION WALLONNE REPRESENTÉE PAR L'EXECUTIF REGIONAL WALLON)</p> <p>* Abstract *</p> <p>--</p>	1, 3	<p>A 61 K 39/00</p> <p>A 61 K 39/44</p>
A	<p>EP - A1 - 0 046 614 (REGION WALLONNE REPRESENTÉE PAR L'EXECUTIF REGIONAL WALLON)</p> <p>* Abstract *</p> <p>--</p>	1, 4, 5, 7	
A	<p>US - A - 4 006 059 (BUTLER)</p> <p>* Claim 1 *</p> <p>----</p>	1, 7, 10	<p>TECHNICAL FIELDS SEARCHED (Int. Cl. 7)</p> <p>C 12 N</p> <p>B 01 J</p> <p>A 61 K</p>
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 12-12-1983	Examiner WOLF
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone</p> <p>Y : particularly relevant if combined with another document of the same category</p> <p>A : technological background</p> <p>O : non-written disclosure</p> <p>P : intermediate document</p> <p>T : theory or principle underlying the invention</p> <p>E : earlier patent document, but published on, or after the filing date</p> <p>D : document cited in the application</p> <p>L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p>			

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